

# Potency of *Lactobacillus plantarum* Dad-13 and Sweet Potato (*Ipomoea batatas*) Fiber as Immunomodulator in Rats Infected With *Salmonella* Typhimurium

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## Abstract

*Lactobacillus plantarum* Dad-13 that isolated from “dadih” (traditional Indonesian fermented milk) has been known as probiotic, while sweet potato fiber has been proven as an effective prebiotic. The objective of this study was to evaluate the potency of *Lactobacillus plantarum* Dad-13 and sweet potato fiber as immunomodulators in terms of intestinal secretory immunoglobulin A (sIgA) and splenocyte gamma-interferon (IFN- $\gamma$ ). Sixty male Sprague Dawley rats (uninfected and infected) were divided into five groups: AIN-93, Indonesian children diet (ICD), Sweet potato fiber (SPF), SPF + *Lactobacillus plantarum* Dad-13 (SPFL), and fructooligosaccharides + Lacto-B (FOSL). After diet intervention, the rats were killed and sampled including intestinal fluid, spleen and caecal digesta. The results showed that soluble fiber such as sweet potato fiber could not increase the number of lactobacilli in infected rats, but could play a role in mucosal immune response through the increasing of sIgA. While, *Lactobacillus plantarum* Dad-13 contained in the combination with sweet potato fiber may has potency in systemic immune stimulation, because of the tendency to increase level of splenocyte IFN- $\gamma$  in infected rats.

**Keywords:** *Lactobacillus plantarum* Dad-13, sweet potato fiber, immunomodulator, lactobacilli, *Salmonella* Typhimurium

## 1. Introduction

The isolate of *Lactobacillus plantarum* Dad-13 from “dadih” was identified using *recA* gene-based multiplex primers (Rahayu, Yogeswara, Tami, & Suparmo, 2011). Traditionally, dadih made from buffalo milk are fermented in a bamboo tube and covered with banana leaves, and then incubated at room temperature (27-33 °C) for 2 d (Sughita, 1985).

The gut microbiota or microflora has a crucial role in human health and disease. The gastrointestinal tract (GIT) is comprised of the entire digestive system from the stomach to the anus. The colon or the large intestine is the organ which is the preferred site for bacterial colonization. Particular changes in the intestinal ecosystem might contribute to the development of certain illness, such as inflammatory bowel disease, antibiotic-associated diarrhea, colon cancer, hypercholesterolemia, and others (Vyas & Ranganathan, 2012).

The human gut is dominated by several bacterial phyla including Bacteroidetes, Firmicutes, and Actinobacteria (Vyas & Ranganathan, 2012). The caecal microbiota was more complex than the jejunal and ileal microbiota. Although facultative anaerobes were also the predominant species in caecal microbiota, obligate aerobes belonging to the Bacteroides group, the *C. coccoides* group and the *C. leptum* subgroup were also present (Hayashi, Takahashi, Nishi, Sakamoto, & Benno, 2005).

The gut immune system is influenced by many factors, including dietary components and commensal bacteria. The composition of commensal bacteria can be influenced by various factors, including host genetics, nutrition, antibiotic treatment, infection, and sequential microbial colonization in the neonatal period. Therefore, prebiotics

and/or probiotics are a powerful strategy for manipulating the microbial composition and immune responses of the host (Vieira, Teixeira, & Martin, 2013).

Gastric juice and bile salt resistant, as well as antagonisms toward pathogenic bacteria were used to screen the probiotic candidates. Based on these criteria, *Lactobacillus plantarum* Dad-13 was included as probiotic. *L. plantarum* Dad-13 was able to inhibit the growth of pathogenic bacteria including *Shigella dysenteriae* dky-4, two strains of pathogenic *Escherichia coli* dky-1 and dky-2, and *Salmonella* Typhimurium dky-3. This strain showed resistance to bile salt (up to 3%), and resistance to gastric juice at pH 2.0-3.0 for 6 h (Rahayu, Yogeswara, Tami, & Suparmo, 2011).

*In vivo* studies showed that *Lactobacillus plantarum* Dad-13 increased total lactobacilli by 1.2 log cycle, but did not reduce the count of *E. coli* and coliforms. Infection of *E. coli* and addition of *L. plantarum* Dad-13 changed the ratio among fecal microbiota of rats (Sumaryati, Utami, & Suparmo, 2009). *L. plantarum* competes with *E. coli* for intestinal colonization in the presence of the mannose-dependent adherence mechanism. This mechanism was partly responsible for the competition with *E. coli* early after colonization, and also influences intestinal and systemic immunity (Herias et al., 1999). The species *Lactobacillus plantarum* 283 is a colonizer of the small human intestine, and *Lactobacillus plantarum* 299 is colonizer in human colon (Johansson et al., 1993). In another study, *Lactobacillus plantarum* Mut7 that had been isolated from fermented cassava possess probiotic criteria such as survival under acidic condition and bile acid, and antagonism against pathogenic bacteria such as *Salmonella choleraesuis* and *Shigella flexneri* (Lestari, Rahayu, & Aman, 2008). Heat-killed *L. plantarum* Mut7 has immunomodulatory effect in human HB4C5 cell line and the capability for increasing the production of IgM was the highest among other indigenous probiotic (Lestari, Harmayani, & Sugahara, 2010). There is limited information on *in vivo* study of immunomodulatory properties of *L. plantarum* Dad-13.

Diarrheal infections caused by *Salmonella*, are one of the major causes of childhood morbidity and mortality in developing countries. *Salmonella* causes various diseases that range from mild gastroenteritis to enteric fever, depending on the serovar involved, infective dose, species, age and immune status of the host (Castillo, Perdigón, & de LeBlanc, 2011). In the previous study, sweet potato fiber extract could increase the lactobacilli population and prevent the *Salmonella* Typhimurium diarrhea. This sweet potato (Bestak variety) containing 11.36% total fiber consisted of 9.23% insoluble fiber and 2.13% soluble fiber (Astuti, 2005). In addition, sweet potato fiber extracts containing fructooligosaccharides (FOS), inulin and raffinose, and showed potency as prebiotic source. This score of prebiotic activity of SPF was similar to FOS, but was higher than inulin (Lestari, Soesatyo Irvati, & Harmayani, 2013). Therefore, the SPF can be developed as functional foods.

Lactobacilli are able to utilise the most commonly used prebiotic oligosaccharides, and the best growth was observed on inulin, followed by lactulose and raffinose (Kunová, Rada, Lisová, Ročková, & Vlková, 2011). According to Gibson and Fuller (2000), preferred target organisms for prebiotics are not only lactobacilli, but also species belonging to the *Bifidobacterium* genera. The most efficient prebiotics may also reduce or suppress numbers and activities of organisms seen as pathogenic. However, contradicting results on prevention of pathogenic *Salmonella* infections with prebiotics have been published. Study by Bruggencate, Bovee-Oudenhoven, Lettink-Wissink, Katan, and Van der Meer (2004), inulin and FOS showed increased salmonella numbers in caecal contents six days after infection. Both inulin and FOS significantly stimulated intestinal colonisation and translocation of salmonella to extraintestinal sites. Additionally, supplementing a cornstarch-based rodent diet with 10% FOS or xyclooligosaccharides (XOS) was found to increase the translocation of *S. Typhimurium* SL1344 to internal organs in mice (Petersen et al., 2009).

Secretory IgA (sIgA) serves as the first line of defense in protecting the intestinal epithelium from enteric toxins and pathogenic microorganisms, through a process known as immune exclusion. This sIgA promotes the clearance of antigens and pathogenic microorganisms from the intestinal lumen by blocking their access to epithelial receptors, entrapping them in mucus, and facilitating their removal by peristaltic and mucociliary activities (Mantis, Rol, & Corthésy, 2011). While, IFN- $\gamma$  and tumor necrosis factor alpha (TNF- $\alpha$ ) cytokines, and also NOS<sub>2</sub> are key elements of cellular immunity (cell-mediated defense) against intracellular pathogens (Parent et al., 2006) such as *Salmonella* Typhimurium (Leung & Finlay, 1991).

The purpose of this study was to evaluate the effect of supplementation of sweet potato fiber and combination of *Lactobacillus plantarum* Dad-13 and sweet potato fiber on total number of caecal lactobacilli and its immunomodulatory properties in rats infected with *Salmonella* Typhimurium.

## 2. Materials and Methods

### 2.1 Bacterial Isolates, Sweet Potato Tuber, Commercial Prebiotic, and Probiotics

Bacterial isolate derived from “dadih” was provided by Food and Nutrition Culture Collection (FNCC) Center for Food and Nutrition Studies, Universitas Gadjah Mada. The sweet potato tuber that was used in this study was Bestak variety from Central Java, Indonesia. The characteristic this tuber were brownish white color in their skin and yellowish white in the interior tuber, spherical and tapered at both ends with diameter was around 8.0 cm. Fructooligosaccharides (FOS) as a commercial prebiotic was provided by Sari Husada Milk Industry (Yogyakarta, Indonesia), whereas the Lacto-B as a commercial probiotic was produced by Novell Pharmaceutical Laboratories (Jakarta, Indonesia). The Lacto-B powder contains three kinds of bacteria (*Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Bifidobacterium longum*) and is known as anti-diarrhea in infant and children.

### 2.2 Preparation of Ethanol-Insoluble Residues From Sweet Potato

Fibers from sweet potato tuber (*Ipomoea batatas*) were prepared according to Mongeau & Brassard (1990) with slight modifications. Whole sweet potato was peeled ( $2 \times 2 \times 2$  cm) and diced prior to extraction, and then steamed for 30 min. The steamed tuber was extracted by using 80% ethanol at 60 °C for 20 min in Waring blender. The warm solutions were filtered by filter paper (Whatman 41), and then the ethanol-insoluble solid was washed with acetone (1:2) for 30 min to produce fluffy white materials. The materials were dried in oven at 50 °C for 10 h and blended.

### 2.3 Experimental Design

Materials in this study consisted of sixty male Sprague Dawley rats three weeks old (were assumed as 1-2 years old children) around 50 g weighed from Laboratory of Integrated Research and Assessment, Universitas Gadjah Mada. Before the treatment of diets, the rats were acclimated for 7 d with standard diet AIN-93G (Reeves, Neilsen, & Fahey, 1993), and given drinking water ad libitum. The rats were divided into five groups: 1) AIN-93, 2) Indonesian children diet (ICD), 3) Sweet potato fiber (SPF), 4) *Lactobacillus plantarum* Dad-13+ SPF (SPFL), and 5) Lacto-B + fructooligosaccharides (FOSL). Each group consisted of 12 rats and was treated with diet for 14 d. Six rats in each group were infected orally with *Salmonella* Typhimurium ( $10^5$  CFU/ml) at day 15 (total 30 rats), and continued to receive the treated diets for 7 d and then killed. The uninfected rats were killed after receiving the diet for 14 d. The caecal, intestinal fluid and spleens of rats were sampled for evaluation of total lactobacilli, sIgA and IFN- $\gamma$ , respectively. The fecal consistency was observed visually in uninfected and infected rats 7 d before and after infection. The lactobacilli were counted by standard plate count (SPC) method. While the intestinal sIgA and IFN- $\gamma$  in spleen lymphocyte culture supernatant, were analyzed by enzyme-linked immunosorbent assay (ELISA) technique. All procedures related to animal experiment were conducted following the recommendation of Medical and Health Research Ethics Committee (MHREC) Faculty of Medicine Universitas Gadjah Mada, Indonesia (number of Ethics Committee Approval: KE/FK/374/EC).

### 2.4 Diets Preparation

Five diets were prepared: 1) Standard diet AIN-93G (Reeves, Neilsen, & Fahey, 1993) as negative control. 2) Indonesian children diet (ICD). The diet composition resembled Indonesian children' food and was made under the recommended dietary allowance for children aged 1-2 years. 3) Sweet potato fiber (SPF) diet. This sweet potato fiber was added (fortified) to standard diet AIN-93. 4) Sweet potato fiber + *Lactobacillus plantarum* Dad-13 diet (SPFL). This sweet potato fiber was added to standard diet AIN-93, whereas the *Lactobacillus plantarum* Dad-13 ( $10^8$  CFU/ml) was administered by oral force feeding. 5) FOS + Lacto-B (FOSL) diet as positive control. FOS was added to the standard diet AIN-93 and Lacto-B ( $10^8$  CFU/ml) was administered by oral force feeding. The composition for five diets is given in Table 1.

Table 1. Composition of standard and intervention diet

Composition	g/kg				
	AIN-93	ICD	SPF	SPFL	FOSL
Casein	200.0	59.3	200.0	200.0	200.0
L-Cystine	3.0	-	3.0	3.0	3.0
Sucrose	100.0	-	100.0	100.0	100.0
Corn starch	529.5	-	529.5	529.5	529.5
Fiber (CMC)	50.0	-	50.0	50.0	50.0
Soy oil	70.0	-	70.0	70.0	70.0
Choline bitartat	2.5	-	2.5	2.5	2.5
AIN-93 Min-Mix	35.0	35.0	35.0	35.0	35.0
AIN-93 Vit-Mix	10.0	10.0	10.0	10.0	10.0
Sweet potato fiber	-	-	32.0	32.0	-
Fructooligosaccharides	-	-	-	-	32.0
<i>L. plantarum</i> Dad-13	-	-	-	10 <sup>8</sup> CFU/ml	-
Lacto-B	-	-	-	-	10 <sup>8</sup> CFU/ml
Rice	-	763.3	-	-	-
Palm oil	-	132.4	-	-	-

### 2.5 Fecal Consistency

Fecal consistency was observed visually in uninfected and infected rats during experiment, by the same individual each morning according to Correa-Matos et al. (2003). The fecal consistency was graded using the following scale: 1 = solid; 2 = semisolid; 3 = loose; 4 = watery. The fecal consistency was observed everyday before and after infection for 7 d.

### 2.6 Total Lactobacilli

Total number of caecal lactobacilli was counted by using standard plate count method on selective medium Rogosa Agar (Jaksevic et al., 2011). Sample of caecal digesta was weighed and serial dilutions were made with physiological salt, and then certain dilutions were inoculated on sterile agar plates. Inoculated agar plate was incubated for 48-72 h at 37 °C, the number of bacterial colony was counted and expressed in log CFU/g digesta.

### 2.7 Intestinal Fluid Collection

Rat intestinal fluid was taken by flushing with 4 ml of cold phosphate-buffered saline (PBS) pH 7 containing 2 mM phenylmethyl sulfonyl fluoride (PMSF) (Sigma), 10 mg tosylphenylalanine chloromethyl ketone (TPCK) (Sigma), 0.02% NaN<sub>3</sub>, and 5 mM ethylene diamine tetra acetic acid (EDTA) (Sigma). Flushing fluid was collected in a sterile of 15 ml conical tubes, then were centrifuged at 926 × g (Sorval, Biofuge primo R), the supernatant was taken and stored at -20 °C until analyzed (Yun, Lillehoj, Shu, & Min, 2000).

### 2.8 Analysis of Intestinal sIgA

Intestinal fluid sIgA was analyzed according to the instructions on the Rat sIgA ELISA Kit (Usen Life Science Inc., Wuhan, China) as follows: 100 µl standard solution, samples and blank were pipetted into a well on the plate, then covered with a plate sealer and incubated at 37 °C for 2 h. The solution was discarded and then added 100 µl solution of detection reagent A (biotin-conjugated polyclonal antibody specific for sIgA) into each well and incubated for 1 h at 37 °C. The solution was removed and then the plate was washed three times with washing solution 1×. Then added 100 µl solution of detection reagent B (Avidin conjugated to Horseradish Peroxidase / HRP) and incubated for 30 min at a temperature of 37 °C. Plate was washed five times, then added 90 µl of substrate solution (tetramethyl benzidine) into each well and incubated for 15-25 min at a temperature of 37 °C in the dark space (fluid will be blue color). Stop solution (sulfuric acid) of 50 µl was added into each well (the liquid will change color to yellow), then read on a microplate reader (Model 680 XR, Bio-RAD) wavelength of 450 nm.

### 2.9 Lymphocyte Culture Supernatant Collection

Isolation and collection procedures of spleen lymphocyte were performed according to Klein, Witonsky, Ahmed, Holladay, and Gogal (2006) with slight modifications. Briefly, spleen was excised aseptically and placed in 10 ml of Rosewell Park Memorial Institute (RPMI)-1640 medium (Sigma) containing 10% FBS (fetal bovine serum: Gibco) and 2% penicillin-streptomycin (Gibco). The spleen was ripped with syringe tip, and also by pipetting up and down and spraying by RPMI medium a few times by using disposable syringe for releasing the lymphocytes. After releasing the lymphocytes from the spleen, the suspension was allowed to separate from cell debris. The supernatant was removed into conical tubes, and then the cells were counted by haemocytometer. The cell concentrations to be cultured were  $5 \times 10^5$  /ml. The lymphocytes were cultured in plate with 96 wells in RPMI medium, and 5 µg/ml of phytohaemagglutinine (PHA) mitogen (Murex) was added to in each well. The volume of the lymphocyte culture was 100 µl in each well. The plate was placed into 5% CO<sub>2</sub> incubator for 72 h at 37 °C. The lymphocyte culture supernatants were removed and analyzed for cytokine IFN-γ production.

### 2.10 Analysis of IFN-γ in Spleen Lymphocyte Culture Supernatant

The amount of IFN-γ contained in rat spleen lymphocyte culture supernatant was measured with specific rat IFN-γ ELISA kit (Bender MedSystem, Vienna, Austria). Briefly, microwell plate was washed twice with wash buffer. The standard was reconstituted with 750 µl aquabidest for Standard of IFN-γ, and the prepared standard was made into a 1:2 dilution in a small tube. For IFN-γ: 225 µl of reconstituted standard IFN-γ (concentration = 4000.0 pg/ml) was pipetted into S1 tube (standard 1) containing 225 µl sample diluent (concentration= 2000.0 pg/ml). This procedure was done for the next tubes until the concentration of the final tube (S7) was 31.3 pg/ml. Each standard (100 µl) was pipetted into well for standard, and 100 µl of sample diluent was pipetted into well for blank. Each well for sample was filled with 50 µl of sample diluent and added 50 µl sample. Biotin conjugate solution was added into microwell plate, and covered with adhesive film. The plate was incubated at room temperature (18-25 °C) for 2 h. Adhesive film was removed, and microwell plate was washed 3 times with 400 µl wash buffer for each well. Streptavidin-HRP solution (100 µl) was added into all well, and covered with adhesive film. The plate was incubated at room temperature for 1 h. Micro plate was washed 3 times and added with 100 µl TMB (tetramethyl-benzidine) substrate to all well. The plate was incubated at room temperature for 10 min, avoiding exposure from direct light. The enzyme reaction was stopped by pipetting 100 µl of stop solution into each well. The absorbance of each microwell was read at a wave length of 405 nm.

### 2.11 Statistical Analysis

The data of total caecal lactobacilli, sIgA and IFN-γ, were analyzed by ANOVA using SPSS 12.0 (2003), Chicago, USA.

## 3. Results and Discussion

### 3.1 Fecal Consistency

The effect of infection with *Salmonella* Typhimurium in fecal consistency in rats that received various diet interventions is shown in Table 2.

Table 2. Fecal consistency of uninfected and rats infected with *Salmonella* Typhimurium

Diets	Uninfected				Infected			
	Solid (1)	Semi solid (2)	Loose (3)	Wattery (4)	Solid (1)	Semi solid (2)	Loose (3)	Wattery (4)
AIN-93	-	-	6	-	-	1	5	-
ICD	6	-	-	-	6	-	-	-
SPF	-	6	-	-	-	3	3	-
SPFL	-	6	-	-	-	1	5	-
FOSL	-	6	-	-	-	4	2	-
Total (n=30)	6	18	6	-	6	9	15	-

Consistency of feces in rats that received Indonesian children diets (ICD) tended to be solid in both in uninfected

and infected rats. The fecal consistency in rats fed sweet potato fiber (SPF), sweet potato fiber + *Lactobacillus plantarum* Dad 13 (SPFL) and fructooligosaccharides + Lacto-B (FOSL) were semi solid in uninfected rats, whereas this consistency in infected rats largely became loose. In the negative control rats (AIN-93), the fecal consistency tended to loose both in uninfected and infected rats. There was no watery consistency in rat fecal of all diets in uninfected or infected rats.

Although the diarrhea prevalence tend to more frequent after salmonella infection, but there was no clear pattern. The rats fed Indonesian children's diet (ICD) had more solid in fecal consistency than the rats fed other diets, and there was no change the fecal consistency in infected rats. This may be due the ICD containing more rice which has less fiber, so that the moisture content was lower. According to Li, Andrews, and Pehrsson (2002), cooked white rice containing 0 soluble fibers, 0.34% insolubel fiber and 0.34% total dietary fiber (TDF).

*Salmonella* Typhimurium is bacteria which causes diarrhea in infant and children, but the effect on rats is not as consistent as in humans. In this study, the uninfected rats fed SPF, SPFL and FOSL had semi solid in fecal consistency, whereas in infected rats, the fecal matter partially became loose in consistency. In the previous study, the rats fed 10% sweet potato fiber showed solid in fecal consistency after infection with salmonella, showing no diarrheal symptoms (Astuti, 2005). This is in accordance with Tauxe and Pavia (1998) which stated that clinically important infections due to *Salmonella* Typhimurium occur only in humans, and humans are the only known reservoir for this organism. Similar to the National Research Council (1991), that *S. enteritidis* serotype Typhimurium is the most common serotype infecting laboratory rodents, although the prevalence of asymptomatic carriers is unknown but probably low. Reports of natural outbreaks of disease are rare in the literature, probably because most infections are asymptomatic in normal hosts. Diarrhea is an uncommon finding. Many host, pathogen, and environmental factors determine the pathologic findings and severity of infection, including host age and genotype; make up of the intestinal flora; nutritional state; immune status; presence of concurrent infections; bacterial serotype; and environmental stressors such as food and water deprivation, temperature, iron deficiency, and experimental manipulations.

### 3.2 Caecal Lactobacilli

There were no significant differences in total lactobacilli in rats that received AIN-93, ICD and FOSL both in uninfected and infected rats. However, the total lactobacilli in infected rats fed SPF and SPFL decreased significantly ( $p < 0.01$ ) compared with uninfected rats. Lactobacilli in rats fed FOSL also decrease in infected rats, even though not significant (Figure 1).

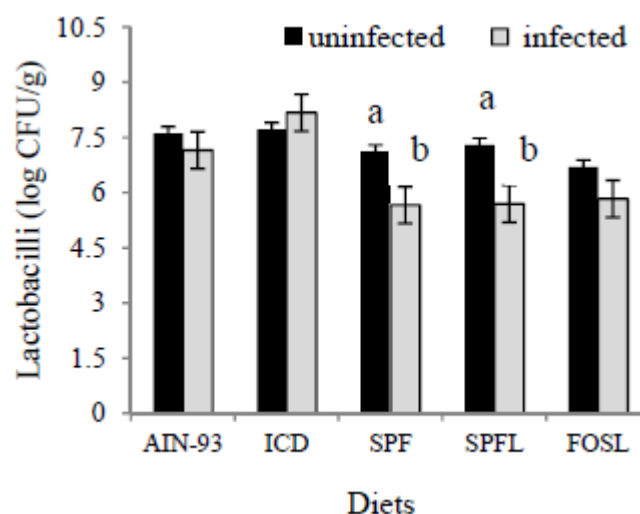


Figure 1. The average of total caecal lactobacilli in uninfected and infected rats fed different diets (AIN-93, ICD, SPF, SPFL, FOSL). Different letters (a, b) indicate significant difference ( $p < 0.01$ ) between uninfected and infected rats in each diet intervention, whereas no letters on top indicate no significant difference

The lower count of lactobacilli in three kinds of diets in this study (SPF, SPFL, FOSL) (Figure 1) indicates these dietary fiber could stimulate the growth of *Salmonella* Typhimurium and then compete with lactobacilli. This effect is likely due to the rapid fermentation of sweet potato fiber and fruktooligosaccharides to production of

metabolites such as SCFA. Inulin and oligofructose are rapidly and completely fermented by the colonic microflora with the production of acetate and other short chain fatty acid (SCFA) (Jenkins, Kendall, & Vuksan, 1999). Rapid production of metabolites results in subsequent irritation and impairment of the mucosal barrier. Total caecal SCFA pools were higher while pH was lower from ingesting oligosaccharide-containing diets compared with control or cellulose diets.

Dietary incorporation of fermentable, indigestible oligosaccharides, by providing SCFA, lowering pH, and increasing bifidobacteria, may be beneficial in improving gastrointestinal health (Campbell, Fahey, & Wolf, 1997). Salmonella can use the SCFA conditions of the mammalian intestinal tract as a signal for invasion. Low total SCFAs (30 mM) with a predominance of acetate induce invasion, whereas high total SCFAs (200 mM) with greater concentrations of propionate and butyrate suppress it (Lawhon, Maurer, Suyemoto, & Altier, 2002). The epithelial cell injury caused by rapid SPF, SPFL and FOSL fermentation in this study cause salmonellae crosses in the distal gut by a paracellular and transcellular route (Kops, Lowe, Bement, & West, 1996). The ability of *Salmonella* Typhimurium to invade the intestinal mucosal cells is an important step in pathogenesis (Durant, Corrier, & Ricke, 2000). *Salmonella* Typhimurium causes enteric and systemic disease by invading the intestinal epithelium of the distal ileum, a process requiring the invasion genes of Salmonella pathogenicity island 1 (SPI-1). The concentration and composition of SCFAs in the distal ileum provide a signal for productive infection by Salmonella, whereas those of the large intestine conditions inhibit invasion (Lawhon, Maurer, Suyemoto, & Altier, 2002). In addition, SCFA may serve as an environmental signal that triggers the expression of invasion genes in the gastrointestinal tract (Durant, Corrier, & Ricke, 2000).

### 3.3 Intestinal sIgA

Demonstrated on Figure 2, only sIgA in infected rats treated with SPF increased significantly ( $p < 0.05$ ) compared with uninfected rats, whereas there were no significant differences in sIgA between uninfected and infected rats in other diets.

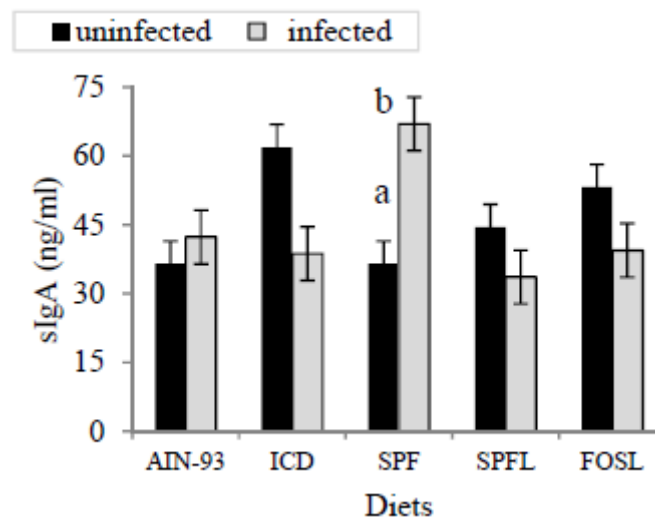


Figure 2. The average of intestinal sIgA in uninfected and infected rats fed different diets (AIN-93, ICD, SPF, SPFL, FOSL). Different letters (a, b) indicate significant difference ( $p < 0.01$ ) between uninfected and infected rats in each diet intervention, whereas no letters on top indicate no significant difference

The increase in level of sIgA in infected compared to uninfected rats fed sweet potato fiber may be due the SPF containing more than one prebiotic namely FOS, inulin and raffinose (Lestari, Soesatyo, Irvati, & Harmayani, 2013). This prebiotics combination may have a better effect on the growth of probiotic and their SCFA production in intestine. In addition, the dietary fiber could act as a more effective prebiotic by inducing major shifts in gut microbial composition and directly affecting the mucosal immune system, resulting in an improvement in enteric inflammatory disorders and the systemic immune response (Vieira, Teixeira, & Martins, 2013). These results are similar to those reported by Bakker-Zierikzee et al. (2006), that formula-fed infants may benefit from infant formulas containing a prebiotic mixture of GOS and FOS because of the observed clear tendency to increase faecal sIgA secretion. Adding viable *Bifidobacterium animalis* strain Bb-12 ( $6.0 \times 10^9$  CFU

/100 ml formula) did not reveal any sign for such a trend. Infants fed on the probiotic formula showed a highly variable faecal sIgA concentration with no statistically significant differences compared with the standard formula group. In this study, the infected rats fed SPFL or FOSL tended to decrease intestinal sIgA compared to uninfected rats, although not statistically different (Figure 2). This indicates that sIgA level is more influenced by prebiotic combination rather than combination of single prebiotic and probiotic.

Contrary to a study by Biedrzycka et al. (2003), young rats receiving more than  $10^9$  Bifidobacterium cells daily for 14 d, have higher bacterial antigen-specific IgA in serum blood compared to the control rats, both in non-infected and Salmonella-challenged animals. This result study by Biedrzycka et al. (2003), similar with the result in other study, that levels of sIgA and lymphocyte proliferation rate were also significantly increased in probiotic dahi-fed mice and infected with *Salmonella enteridis* compared with mice fed milk and control dahi. (Jain, Yadav, & Sinha, 2009). This different results from this study may be due to differences in the probiotic bacterial strain and dose, and also experimental animal used.

Most dietary fibers that have fermentable carbohydrates could be considered as prebiotics as well. There is a hypothesis that any type of dietary or food supplement that could promote the growth of beneficial bacteria and consequently promote homeostasis in the gut and good health could be considered as a prebiotic, even though the supplement may not meet the required criteria. Prebiotics and/or probiotics are a powerful strategy for manipulating the microbial composition and immune responses of the host (Vieira, Teixeira, & Martins, 2013).

SCFA production, particularly butyrate, in the colon may reduce the requirement of epithelial cells for glutamine, thereby sparing it for other cells, such as those of the immune system. This is possibly as a result of its ability to spare glutamine as a substrate for the colonic mucosa by provision of increased SCFA. Because glutamine is a preferred substrate for lymphatic tissue, it is possible that this may improve immune function under some circumstances. Such an effect may also be relevant to inulin and oligofructose if SCFA production is increased in the proximal, even if not in the distal colon (Jenkins & Kendall, 1999). This hypothesis is supported by the observation that lactulose administration can increase serum glutamine levels (Jenkins, & Kendall, 1997). Glutamine is an important energy source for immune lymphocytes, because glutamine at near-physiological concentration can be readily utilized by these cells (Wu, Field, & Marliss, 1991) for rapidly dividing cells such as lymphocytes and epithelial cells of intestinal mucosa (Windmueller, 1982). It is well established that the fermentation of inulin and oligofructose increases the production of SCFA, primarily acetate, butyrate and propionate in the gut (Gibson & Roberfroid, 1995). Nevertheless, a number of studies support that SCFA improve components of non specific immune responses (Pratt, Tappenden, McBurney, & Field, 1996). Modulation of T-cell responses by n-butyrate could also result from altered antigen-presenting cells (APC) function, possibly as a consequence of downregulating distinct adhesion and/or costimulatory receptors as well as of inducing apoptosis. A potential clinical relevance of SCFA for reducing T-cell-mediated immune reactions via modulating APC function is speculated (Bohmig et al., 1997).

#### 3.4 IFN- $\gamma$ in Spleen Lymphocyte Culture

The level of IFN- $\gamma$  in infected rats fed SPF was lower ( $p < 0.05$ ) than uninfected rats, same as in the ICD diet. In the AIN-93 diet, the IFN tended to decrease in infected rats, even though the decrease was not significant. However, the level of IFN- $\gamma$  in infected rats fed SPFL and FOSL diets increased compared with uninfected rats, even though the increase was not significantly different (Figure 3).



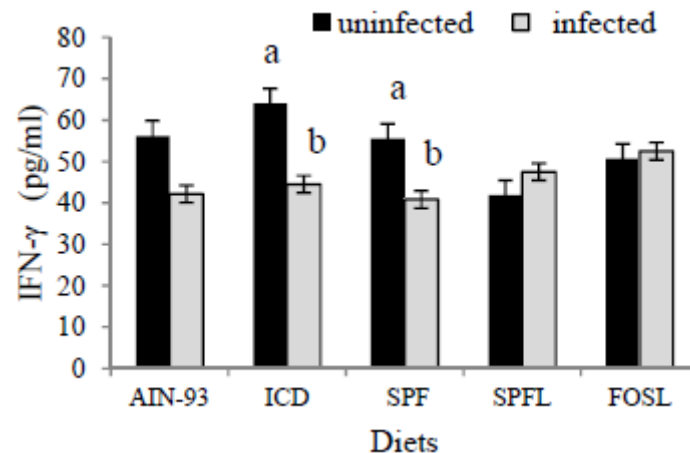


Figure 3. The average of IFN- $\gamma$  in spleen lymphocyte culture in uninfected and infected rats fed different diets (AIN-93, ICD, SPF, SPFL, FOSL). Different letters (a, b) indicate significant difference ( $p < 0.05$ ) between uninfected and infected rats in each diet intervention, whereas no letters on top indicate no significant difference

Even though not significant, the increasing IFN- $\gamma$  in infected rats fed SPFL and FOSL indicate that stimulation for this cytokine needs probiotic, because in infected rats fed SPF the cytokine decreased significantly (Figure 3). No significant increase in IFN- $\gamma$ , may be caused of the low doses of probiotic *Lactobacillus plantarum* Dad-13 in SPFL diet and also Lacto-B in FOSL diet. In the previous study by Yuan, Wen, Liu, and Li (2013), the dose effects of *Lactobacillus acidophilus* on IFN- $\gamma$  producing T cell was similar between the intestinal and systemic lymphoid tissues. These findings have significant implications in the use of probiotic lactobacilli as immunostimulatory versus immunoregulatory agents. Therefore, that probiotics can be ineffective or even detrimental if not used at the optimal dosage for the appropriate purposes, highlighting the importance of not only strain but also dose selection in probiotic studies. According to Vinderola, Matar, and Perdigon (2005), that probiotic-bacteria-intestinal epithelial cell (IEC) interaction, which releases signals from the IEC, could play a major role in the innate immune response induced by lactic acid bacteria (LAB), depending on the dose administered (Galdeano, de LeBlanc, Bonet, & Perdigon, 2007). As the induce cytokine profiles and intrinsic adjuvanticity properties by LAB was also strain-dependent (Maassen et al., 2000).

Since the increasing of splenocyte IFN- $\gamma$  in infected rats fed SPFL diet was higher than FOSL diet (commercial probiotic), this means *Lactobacillus plantarum* Dad-13 which is the local isolate, has potency as immunomodulator in systemic immune response. In the previous study, probiotic dahi augmented lymphocyte proliferation and enhanced T-cell response towards Th1 by stimulating the production of IL-2, IL-6 and IFN- $\gamma$ . This twist in immune response may help to eradicate *S. enteritidis* infection (Jain, Yadav, Sinha, Naito, & Marotta, 2008).

After interaction between probiotic bacteria and the immune cells in the Peyer's patches (PP), the probiotic bacteria or their fragments are internalized by M cells or in a paracellular way through follicle-associated epithelial cells of the PP. After that, the bacteria or their particles interact with the macrophages and dendritic cells, which are activated to produce cytokines. As consequence of the bacterial stimulation to the immune cells in this inductor site of the immune response, cytokine production is enhanced. The cytokines released by probiotic stimulation in PP are the biological messengers of the complex network of signals that activate the systemic immune response (Galdeano, de LeBlanc, Bonet, & Perdigon, 2007).

The functions of IFN- $\gamma$  have classically focused on the interactions of macrophages and CD4<sup>+</sup> T cells. The interaction of a T-cell receptor with an antigen bound to a major histocompatibility complex (MHC) molecule triggers production of IFN- $\gamma$  by T cells. This IFN- $\gamma$  then acts to activate macrophages, up-regulating a number of gene products and rendering macrophages additionally cytotoxic by increasing oxidative burst and the production of other oxidants such as nitric oxide. Recently, IFN- $\gamma$  was shown to be produced by a number of other immune cell types, including natural killer (NK) cells and macrophages; and to regulate the functions of many of these cell types (Ellis & Beaman, 2004).

#### 4. Conclusions

To induce a systemic immune response associated IFN- $\gamma$  production in rat infected with *Salmonella* Typhimurium, probiotics such as *Lactobacillus plantarum* Dad-13 influenced more than prebiotic contained in sweet potato fiber. Nevertheless, fiber components contained in the sweet potato has a significant role in the mucosal immune system through the increasing of sIgA in rat with *Salmonella* Typhimurium, although there was no increase in caecal lactobacilli. This research strengthens the evidence of previous studies, which reported that rapid fermented fiber increased salmonella translocation, thus decreased lactobacilli population in intestine. Further research is required to study the effect of various doses of *L. plantarum* Dad-13, and various combinations of local prebiotic sources with different levels of solubility on stimulation of mucosal and systemic immune responses in rats infected. The microbial diversity of rat caecum is an interesting subject for further study and need to be analyzed using molecular technique.

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